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# **<sup>13</sup>C MR Hyperpolarization of Lactate by Using ParaHydrogen and Metabolic Transformation in Vitro**

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1619402> since 2016-11-30T23:36:43Z

*Published version:*

DOI:10.1002/chem.201605329

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(Article begins on next page)

# Chemistry - A European Journal

## 13C-MR Hyperpolarization of Lactate using ParaHydrogen and metabolic transformation in vitro.

--Manuscript Draft--

<b>Manuscript Number:</b>	
<b>Article Type:</b>	Full Paper
<b>Corresponding Author:</b>	Francesca Reineri, Ph.D. University of Torino torino, ITALY
<b>Corresponding Author E-Mail:</b>	francesca.reineri@unito.it
<b>Order of Authors (with Contributor Roles):</b>	Eleonora Cavallari Carla Carrera Silvio Aime Francesca Reineri, Ph.D.
<b>Keywords:</b>	magnetic resonance, metabolism, hyperpolarization, cancer
<b>Manuscript Classifications:</b>	Cancer; Imaging agents; Metabolism; NMR spectroscopy
<b>Suggested Reviewers:</b>	Edward Chekmenev eduard.chekmenev@vanderbilt.edu  Jan Bernd Hoevener Jan.Hoevener@uniklinik-freiburg.de  Dean Sherry dean.sherry@utsouthwestern.edu
<b>Opposed Reviewers:</b>	
<b>Abstract:</b>	Hyperpolarization of the <sup>13</sup> C magnetic resonance signal of L-[1- <sup>13</sup> C]Lactate has been obtained using the chemically based, cost-effective method named ParaHydrogen Induced Polarization by means of Side Arm Hydrogenation (PHIP-SAH). Two ester derivatives of lactate have been tested and the factors that determine the polarization level on the product have been investigated into details. The metabolic conversion of hyperpolarized L-[1- <sup>13</sup> C]Lactate into Pyruvate has been observed in vitro using lactate dehydrogenase (LDH) and in a cells lysate. From the acquisition of a series of <sup>13</sup> C-NMR spectra, the metabolic build-up of the [1- <sup>13</sup> C]Pyruvate signal has been observed. These studies demonstrate that, even if the experimental set-up used for these PHIP-SAH hyperpolarization studies is still far from optimal, the attained polarization level is already sufficient to carry out in vitro metabolic studies.
<b>Author Comments:</b>	Dear editor, enclosed please find the manuscript entitled "13C-MR Hyperpolarization of Lactate using ParaHydrogen and metabolic transformation in vitro " to be considered for publication in Chemistry, a European Journal. A previous version of the manuscript had been submitted to this journal and one of the two referees evaluated it a highly important manuscript, while the other considered it too specialized for publication, than the manuscript was rejected. Nevertheless, the editor suggested that an appropriately supplemented manuscript should be reconsidered. Here is submitted a deeply revised version of the manuscript. New experiments have been added, in accordance with the reviewers' suggestions, and new results have been obtained. The whole text has been extensively edited and integrated. We hope that, with the integration of the new results, the manuscript will be considered worth of publication in Chemistry. Yours sincerely

	Francesca Reineri
<b>Section/Category:</b>	
<b>Additional Information:</b>	
<b>Question</b>	<b>Response</b>
Dedication	
Submitted solely to this journal?	Yes
Has there been a previous version?	Yes
Please state previous 1) Manuscript ID and 2) journal. 3) If the paper was reviewed, please include a point-by-point response to the reviewer comments. as follow-up to "Has there been a previous version?"	chem.201604322 Chemistry, a European Journal  the response to reviewers' comments is added as "additional informations"
Do you or any of your co-authors have a conflict of interest to declare?	No. The authors declare no conflict of interest.
Animal/tissue experiments?	No

# <sup>13</sup>C-MR Hyperpolarization of Lactate using ParaHydrogen and metabolic transformation *in vitro*.

Dr. E. Cavallari,<sup>[a]</sup> Dr. C. Carrera,<sup>[a]</sup> Prof. S. Aime,<sup>[a,b]</sup> Dr. F. Reineri <sup>\*[a]</sup>

**Abstract:** Hyperpolarization of the <sup>13</sup>C magnetic resonance signal of L-[1-<sup>13</sup>C]Lactate has been obtained using the chemically based, cost-effective method named ParaHydrogen Induced Polarization by means of Side Arm Hydrogenation (PHIP-SAH). Two ester derivatives of lactate have been tested and the factors that determine the polarization level on the product have been investigated into details. The metabolic conversion of hyperpolarized L-[1-<sup>13</sup>C]Lactate into Pyruvate has been observed *in vitro* using lactate dehydrogenase (LDH) and in a cells lysate. From the acquisition of a series of <sup>13</sup>C-NMR spectra, the metabolic build-up of the [1-<sup>13</sup>C]Pyruvate signal has been observed. These studies demonstrate that, even if the experimental set-up used for these PHIP-SAH hyperpolarization studies is still far from optimal, the attained polarization level is already sufficient to carry out *in vitro* metabolic studies.

## Introduction

The observation that the parahydrogen spin order can be exploited to obtain magnetic resonance hyperpolarized signals is a relatively recent one.<sup>[1]</sup> Since the early studies, the application of this easy to handle and cost effective hyperpolarization methodology evolved quickly to the first *in vivo* studies carried out using <sup>13</sup>C hyperpolarized tracers,<sup>[2]</sup> for which more than 20% hyperpolarization was claimed. The parahydrogen spin order is transformed into MR hyperpolarization when the two protons are added pairwise to an unsaturated bond so that their chemical equivalence is lost. On this basis, the applicability of parahydrogen as hyperpolarization tool appeared to be limited to molecules for which a de-hydrogenated precursor would have been available.

Conversely, dissolution-Dynamic Nuclear Polarization (d-DNP)<sup>[3]</sup> is a hyperpolarization method that does not suffer from limitations regarding the hyperpolarization substrates, albeit it requires sophisticated and expensive instrumentation. The possibility to obtain hyperpolarized metabolites by means of DNP gathered a great deal of interest in the investigation of metabolic transformations *in vivo*.<sup>[4]</sup> It has been suggested that monitoring the metabolic signatures of <sup>13</sup>C hyperpolarized molecules may allow to discriminate healthy from malignant

tissues,<sup>[5]</sup> to probe the disease aggressiveness and early detect the response to treatment.<sup>[6]</sup> Thanks to their potential clinical role, these studies quickly evolved from the first *in-vivo* studies to pre-clinical research and clinical trials.<sup>[7]</sup>

The availability of this powerful tool for metabolic investigation is strongly limited by the high cost of the DNP polarizer. The recent introduction of the so called PHIP-SAH strategy<sup>[8]</sup> allowed to significantly extend the applicability of parahydrogen to metabolites (e.g. to pyruvate) that were before not amenable to this hyperpolarization method. The SAH strategy relies on the functionalization of the molecule of interest (a carboxylate containing molecule) with an unsaturated alcohol (the "side arm"). The unsaturated bond on the alcoholic moiety of the ester is hydrogenated using para-enriched hydrogen and the non-equilibrium spin states population is transferred from parahydrogen protons to the <sup>13</sup>C carboxylate by means of the application of a magnetic field cycle (MFC).<sup>[9]</sup> In practice, in PHIP-SAH, the <sup>13</sup>C atom is hyperpolarized by the parahydrogen protons three and four (or four and five) bonds away using <sup>3</sup>J<sub>H-13C</sub> and <sup>4</sup>J<sub>H-13C</sub> (or <sup>4</sup>J<sub>H-13C</sub> and <sup>5</sup>J<sub>H-13C</sub>) instead of <sup>2</sup>J<sub>H-13C</sub> and <sup>3</sup>J<sub>H-13C</sub> in traditional PHIP approach.

In principle, one may think of the use of hyperpolarized esters in *in vivo* studies, as it has been reported in brain studies, thanks to the ability of neutral esters to cross the lipophilic blood-brain barrier.<sup>[10]</sup> A more general approach relies on the removal of the hydrogenated side-arm by means of hydrolysis to obtain the free carboxylate containing compound.

PHIP-SAH methodology is currently in its infancy and needs optimization of various steps in order to improve the final level of attainable polarization. However it may represent a real breakthrough in the field of metabolic imaging studies since the cost effective, easy to implement parahydrogen based hyperpolarization method would contribute to a much wider applicability of <sup>13</sup>C MR hyperpolarized probes to metabolic imaging studies.

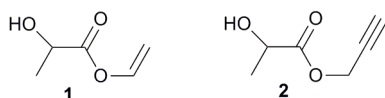
Although most of the metabolic studies using <sup>13</sup>C hyperpolarized tracers have been carried out using DNP polarized [1-<sup>13</sup>C]Pyruvate, it has been shown that L-[1-<sup>13</sup>C]Lactate may offer some advantages.<sup>[11]</sup> Pyruvate and lactate are mutually converted by the lactate dehydrogenase enzyme (LDH) and a large pool of lactate is usually observed in tumours compared to that of pyruvate. Therefore, lactate can be injected at nearly physiological concentration (in the mM range)<sup>[11]</sup> while the concentration of hyperpolarized pyruvate used in these experiments has to be much higher than the physiological values. The lactate derivative 1-<sup>13</sup>C phospholactate has been previously hyperpolarized using parahydrogen<sup>[12]</sup> showing a good performance also *in vivo* experiments.<sup>[13]</sup> Herein we show that it is possible to go straight to <sup>13</sup>C hyperpolarized L-lactate by the PHIP-SAH route.

[a] Department of Molecular Biotechnology and Health Sciences  
University of Torino  
via Nizza 52, Torino, Italy  
E-mail: francesca.reineri@unito.it

[b] Istituto Bioimmagini e Biostrutture del CNR  
Sezione di Torino c/o Center of Molecular Imaging  
Via Nizza 52, Torino, Italy

## Results and Discussion

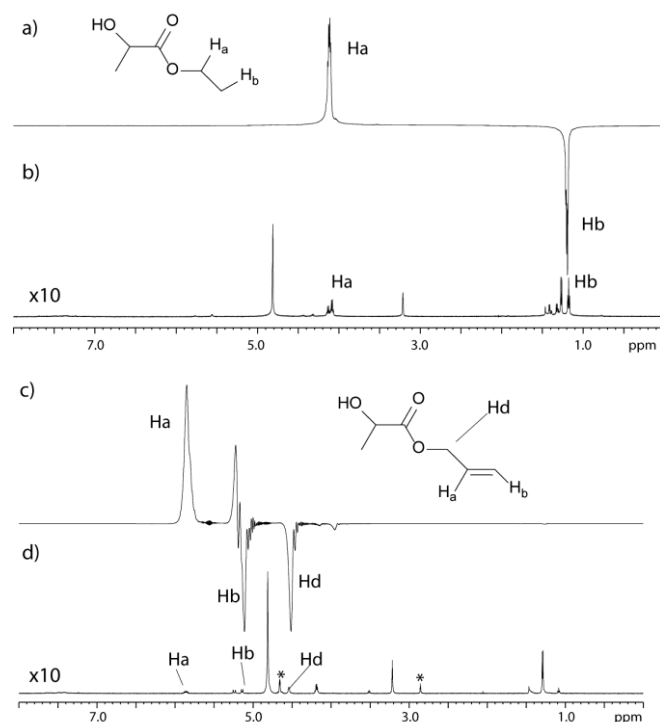
Two esters, vinyl and propargyl L-lactate (scheme 1) have been synthesized and reacted with parahydrogen under the same conditions in order to test the hydrogenation yields and the  $^1\text{H}$  hyperpolarization. Hydrogenation of the substrates (80mM methanol- $d_4$  solution) has been carried out in NMR tubes, using  $[\text{RhCOD}(\text{ddpb})][\text{BF}_4]$  (5mM) as catalyst (see S.I. for details).



**Scheme 1.** Ester derivatives of L-lactate: 1) vinyl lactate; 2) propargyl lactate.

 $^1\text{H}$  HYPERPOLARIZATION

Whereas the complete transformation of the vinyl to ethyl ester has been observed, the hydrogenation of the triple bond proceeds, in part, to the full reduction of the alcoholic moiety. This is in accordance with the expectations that the hydrogenation of a triple bond is easier than that of a double one, therefore higher concentrations of the substrate have to be used under the applied reaction conditions (figure 1c and 1d).



**Figure 1.** a)  $^1\text{H}$ -NMR hyperpolarized spectrum obtained from the ALTADENA hydrogenation of 1 and b) after relaxation. c)  $^1\text{H}$ -NMR after ALTADENA hydrogenation of 3 and d) at thermal equilibrium. Some residual substrate can be observed (peaks indicated with the asterisk) since higher amount of substrate was used (100mM) in order to prevent the formation of the completely hydrogenated alcohol.

In order to evaluate the efficiency of singlet state transfer from parahydrogen to the product, ALTADENA experiments

have been carried out and  $^1\text{H}$  NMR spectra have been acquired. In these experiments, the parahydrogen spin order is converted into  $^1\text{H}$  net magnetization during the adiabatic passage from earth's field, where the hydrogenation reaction occurs, to the high field of the NMR magnet.<sup>[14]</sup> It has been observed (table 1) that, upon parahydrogenation of the vinyl ester, the polarization on the aliphatic signals is 2.6 -3.4%. If the propargyl ester is hydrogenated using parahydrogen, the olefinic signal  $H_a$  shows 7% polarization while 3% is obtained on the methylenic protons ( $H_d$  protons). This observation is also consistent with the fact that triple bonds are hydrogenation faster than double, and brings the important caveat that hydrogenation intermediates for substrate 2 have shorter lifetimes. As a consequence the singlet-triplet mixing on intermediates, i.e. mixing between the pure singlet state of parahydrogen with the triplet state, has a smaller effect on the polarization level of the product.<sup>[15]</sup>

In order to have some clue about the polarization loss that occurs during the hydrogenation reaction, the parahydrogen percentage in the NMR tubes has been measured, after hydrogenation, according to the method described by Feng et al. (details in the S.I.).<sup>[17]</sup> It has been observed that, after that the parahydrogenation reaction is completed, the parahydrogen percentage left in the NMR tubes is about 45-50% thus indicating that a large percentage of parahydrogen is converted to orthohydrogen. Therefore the maximum theoretical polarization is considerably lowered from the initial 90% (with 92% para enrichment) to about 30%. The parahydrogen relaxation might be due to the interaction of the  $\text{H}_2$  molecules with paramagnetic impurities in the hydrogenation mixture. The acquisition of an EPR spectrum allowed to clearly identify the presence of paramagnetic species (see S.I.). Those impurities might derive from partial catalyst degradation, therefore use of lower concentration and more stable catalysts may allow to reduce these species. Furthermore, the application of a reactor in which the hydrogenation mixture (catalyst and substrate) is sprayed into a large parahydrogen volume<sup>[18]</sup> would also minimize parahydrogen relaxation and increase the hyperpolarization on products.

 $^{13}\text{C}$  HYPERPOLARIZATION

Hyperpolarization of the  $^{13}\text{C}$  carboxylate signal depends on the efficiency of singlet state addition to the product and on the efficiency of polarization transfer from parahydrogen to  $^{13}\text{C}$ . It has been shown that magnetic field cycle (MFC) allows this polarization transfer even in the case of small J couplings between parahydrogen protons and the target  $^{13}\text{C}$ .<sup>[9a]</sup> Following to the application of the same MFC profile to the vinyl and to the propargylic ester, the  $^{13}\text{C}$  hyperpolarization is the same (2-2.5%) on the two products. One may note that the polarization of protons that are three bonds away from the carboxylate, i.e. the aliphatic protons  $H_a$  of ethyl ester (3.4%) and the methylenic protons of the allyl ester ( $H_d$ , 3%), is almost completely transferred to the  $^{13}\text{C}$  carboxylate. Conversely, hyperpolarization of the vinyl proton  $H_a$  of the allyl ester, that is coupled to the  $^{13}\text{C}$  carboxylate through a  $^4\text{J}$  coupling, is higher (7%). On the basis of this observation, the MFC profile has been modified in order to facilitate the polarization transfer through smaller J couplings and further increase the polarization level on the  $^{13}\text{C}$  signal of the propargylic ester.



**Table 1.**  $^1\text{H}$  polarization observed in ALTADENA experiments upon parahydrogenation of **1** and **2** and  $^{13}\text{C}$  polarization after the application of MFC.

	$\text{H}_a$	$\text{H}_b$	$\text{H}_d$	$^{13}\text{C}$
Ethyl-lactate	$3.4 \pm 0.1\%$	$2.6 \pm 0.1\%$	-	$2.0 \pm 0.1\%$ [a]
Allyl-lactate	$7 \pm 0.5\%$	$4 \pm 0.5\%$	$3 \pm 0.5\%$	$2.5 \pm 0.5\%$ [a]
Allyl lactate				$4.5 \pm 0.5\%$ [b]

[a]  $^{13}\text{C}$  hyperpolarization obtained using the same magnetic field cycle (exponential remagnetization) on both esters and [b] using slower remagnetization on allyl ester ( $^{13}\text{C}$ -NMR in the SI).

It must be considered that the  $J$  coupling between  $\text{H}_a$  (on the allyl ester) and the  $^{13}\text{C}$  carboxylate nucleus is  $<1\text{Hz}$  while the corresponding  $J$  with the methylenic protons is  $3.2\text{Hz}$ . Since  $^1\text{H}$ - $^{13}\text{C}$  isotropic mixing is achieved when  $\Delta\nu_{\text{H-C}} \sim J_{\text{H-C}}$ , the passage between strong to weak coupling that occurs during the adiabatic passage is achieved at lower magnetic field in the case of the allyl proton  $\text{H}_a$  in respect to the ethyl or methylene ones. Furthermore, for the adiabaticity criterion,<sup>[19]</sup> the minimal time compatible with adiabatic switching, for an AB two spin system, is roughly given by  $1/J_{\text{AB}}$ , therefore the remagnetization passage must be slowed down in order to facilitate the polarization transfer from the olefinic proton  $\text{H}_a$  to the target  $^{13}\text{C}$  carboxylate. On this basis, the remagnetization step has been carried out between  $25\text{nT}$  ( $\Delta\nu_{\text{H-C}} \sim 0.8\text{Hz}$ ) and  $500\text{nT}$  ( $\Delta\nu_{\text{H-C}} \sim 16\text{Hz}$ ) in  $5''$ . It has been experimentally found that, when the passage from  $25\text{nT}$  to  $500\text{nT}$  occurs in  $5''$ ,  $^{13}\text{C}$  polarization is increased to  $4.5\%$ .

### $^{13}\text{C}$ HYPERPOLARIZED L-[1- $^{13}\text{C}$ ]LACTATE

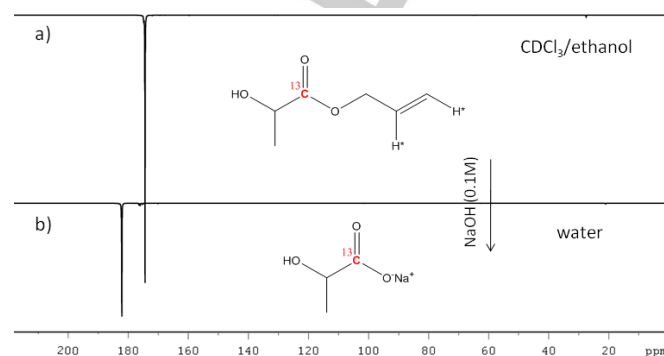
The use of vinyl lactate would allow to obtain an ethyl ester, that is more bio-compatible than the allyl ester derived from hydrogenation of the propargyl derivative. Hepatotoxicity of allyl esters has been shown<sup>[20]</sup> under repeated oral administration of allyl acetate  $100\text{mg/kg}$  in rats ( $\text{LD}_{50}$  in rats  $130\text{mg/Kg}$ ), therefore the use of vinylated esters is more advisable.<sup>[21]</sup>

In the present work, on the basis of the observed polarization, propargyl lactate has been chosen for the generation of hyperpolarized L-[1- $^{13}\text{C}$ ]Lactate.

In order to obtain a hyperpolarized metabolite suitable for in vivo studies, an aqueous solution is needed, without the hydrogenation complex. The use of a supported catalyst would allow to obtain complete removal of the toxic metal from the solution of the hyperpolarized product and to recover the expensive catalyst, and the development of these catalysts will be an important step towards the clinical translation perspective.<sup>[22]</sup> In this work, the phase transfer method has been applied.<sup>[23]</sup> According to this procedure, the lipophilic substrate is hydrogenated in an organic, hydrophobic solvent, (e.g. chloroform/methanol or chloroform/ethanol mixture 5/1). Then an aqueous base (NaOH) is quickly added to the organic phase in order to attain the hydrolysis of the ester and the extraction of the hydrophilic carboxylate-containing compound in the aqueous phase, while the metal complex is retained in the organic phase.

Hydrolysis of the allyl ester has been obtained using a aqueous solution of sodium hydroxide (NaOH  $100\text{mM}$ ) and sodium ascorbate ( $50\text{mM}$ ). The latter acts as a scavenger of paramagnetic impurities.<sup>[24]</sup> Using a homemade set-up, the aqueous base has been pressurized ( $3\text{bar}$ ), heated ( $350\text{K}$ ) and

injected into the NMR tube containing the hyperpolarized ester. An emulsion of the two phases is instantaneously formed and, following to the addition of an acidic buffer (HEPES  $100\text{mM}$ , pH 6), the physiological pH has been reached (pH  $7 \pm 0.5$ ). The separation of the two phases is quick ( $3-4''$ ). Then the upper aqueous solution, containing the hyperpolarized metabolite ( $60 \pm 10\text{mM}$ ), has been transferred into a NMR tube for the acquisition of the  $^{13}\text{C}$ -NMR spectra (figure 2).



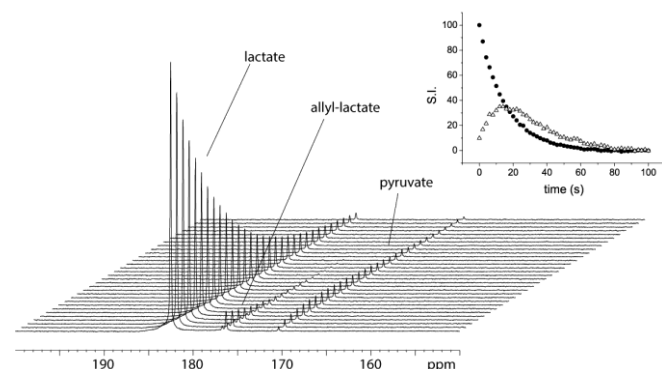
**Figure 2.** a)  $^{13}\text{C}$ -NMR spectrum of  $^{13}\text{C}$  labelled **2** after hydrogenation with parahydrogen and application of magnetic field cycle (ethanol/ $\text{CDCl}_3 = 1/5$ ); b)  $^{13}\text{C}$ -NMR of hyperpolarized L-[1- $^{13}\text{C}$ ]-Lactate obtained after the hydrolysis of the ester.

The polarization level observed on the  $^{13}\text{C}$  signal of the product is  $2.9 \pm 0.2\%$  (corresponding to S.E.  $2400 \pm 200$  at  $14.1\text{T}$ ) and the concentration is  $50 \pm 10\text{mM}$  (see figure S9). Overall, the hydrolysis work-up takes approximately  $40\text{s}$  during which a substantial loss of polarization takes place (hyperpolarization decays towards the thermal equilibrium with a rate dictated by the  $T_1$  of the [1- $^{13}\text{C}$ ]lactate signal in the aqueous solution). Being the whole process carried out manually, this time might be dramatically reduced, e.g. to  $10-20''$ , when a dedicated automatized set-up will be available. In order to be able to back-calculate the initial  $^{13}\text{C}$  hyperpolarization, the longitudinal relaxation time constant ( $T_1$ ) at earth's field has been evaluated. The time delay after hydrolysis ( $\Delta t$ ) has been gradually increased and  $^{13}\text{C}$  signal enhancement has been measured on different samples, at increasing  $\Delta t$  (see S.I.). It is found that  $T_1 = 40 \pm 1\text{s}$  and the polarization level, back-calculated at time zero, is  $7.9 \pm 0.4\%$ .

### METABOLIC TESTS

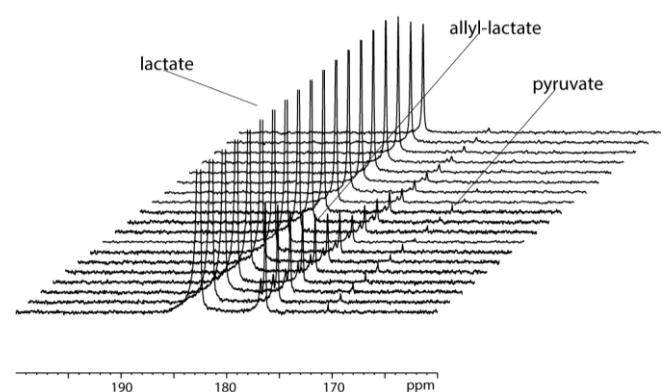
The metabolic transformation of the hyperpolarized L-[1- $^{13}\text{C}$ ]Lactate into [1- $^{13}\text{C}$ ]Pyruvate has been tested using a buffered solution of LDH, NAD $^+$ /NADH and Sodium pyruvate. The enzyme solution has been placed into a  $10\text{mm}$  NMR tube inside the NMR spectrometer ( $14.1\text{T}$ ), with one connecting inlet line for the perfusion of the hyperpolarized metabolite, and kept at  $310\text{K}$ . After hyperpolarization and hydrolysis of the ester, part of the aqueous phase ( $150\mu\text{l}$ ) has been collected into a syringe, diluted with a buffered solution of pyruvate ( $350\mu\text{l}$  HEPES solution, final concentration of pyruvate  $1\text{mM}$ ) and injected, through the inlet line, into the enzyme-containing solution. After mixing of the hyperpolarized metabolite a series of small flip angle  $^{13}\text{C}$  NMR spectra has been acquired from which the metabolic build-up of [1- $^{13}\text{C}$ ]pyruvate can be clearly observed

(figure 3). The concentration of L-[1-<sup>13</sup>C]Lactate in the final solution was determined (4±1 mM) by adding <sup>13</sup>C-enriched urea (3mM) as an internal reference (see figure S10).



**Figure 3.** Series of <sup>13</sup>C-NMR spectra (~15° pulses) acquired following to the perfusion of [1-<sup>13</sup>C]lactate, hyperpolarized by means of PHIP-SAH, to an aqueous solution of LDH (HEPES solution containing NAD<sup>+</sup>/NADH). b) time-dependent signal intensity of [1-<sup>13</sup>C]lactate (filled dots) and [1-<sup>13</sup>C]pyruvate (open triangles).

The possibility of following metabolic transformation has also been tested on breast cancer (TS/A)<sup>[25]</sup> lysed cells. Cells (2×10<sup>7</sup>) have been harvested from the growing plate, washed once with their growing medium and lysed. Then the growing medium (1ml) has been added, the solution has been transferred in the NMR tube and the same experiment used for the enzyme-containing solution has been carried out. A low level of [1-<sup>13</sup>C]Pyruvate can be detected, which is consistent with what observed using DNP hyperpolarized lactate.<sup>[11]</sup> In fact the sensitivity of the measurement of LDH activity obtained from the exchange between L-[1-<sup>13</sup>C]lactate and endogenous pyruvate is much smaller than that of the exchange between [1-<sup>13</sup>C]pyruvate and endogenous lactate.<sup>[11b]</sup> However the advantages associated to the use of lactate for in vivo studies have been demonstrated using H/D exchange on L-[1-<sup>13</sup>C, U-<sup>2</sup>H]Lactate.<sup>[11b]</sup>



**Figure 4.** Series of <sup>13</sup>C-NMR spectra (~15° pulses) acquired following to the perfusion of [1-<sup>13</sup>C]lactate, hyperpolarized by means of PHIP-SAH, to a cells lysate (20×10<sup>6</sup> TSA cells) dissolved in the cells grow medium (1ml RPMI).

## Conclusions

An aqueous solution of hyperpolarized L-[1-<sup>13</sup>C]Lactate, suitable for in vitro metabolic studies, has been obtained by means of the PHIP-SAH method. The potential main factors that determine the polarization level on the end product have been investigated, namely the efficiency of the singlet state transfer to the hydrogenation product and the polarization transfer from parahydrogen protons to the target <sup>13</sup>C carboxylate signal. It has been observed that the polarization level on protons, in ALTADENA experiments, is significantly lower than the theoretical maximum expected using 90% enriched parahydrogen. In separate experiments, parahydrogen relaxation in the NMR tube, following to hydrogenation, has been measured and it has been observed that a large part of parahydrogen is converted into orthohydrogen during the hydrogenation reaction in the NMR tube. This phenomenon is likely due to the interaction between parahydrogen and paramagnetic impurities in the hydrogenation mixture. A considerable increase of the polarization level can be predicted, following to removal of these radicals and use of a hydrogenation reactor. A tailored magnetic field cycle profile has been used in order to increase the polarization transfer from the most polarized proton of the propargyl derivative to the target <sup>13</sup>C carboxylate signal. This allowed to observe  $P_{13C} = 4.8\%$  on the parahydrogenated allyl-lactate. Albeit the polarization level is not optimized yet, it has been shown that it is well sufficient for carrying out in vitro metabolic studies.

## Experimental Section

Hydrogenation reactions have been carried out into NMR tubes equipped with gas-tight teflon valve. Before hydrogenation of the substrate, the catalyst [1,4-Bis(diphenylphosphino)butane](1,5-cyclooctadiene)Rh(I) tetrafluoroborate (1mmol, Sigma-Aldrich, CAS 70255-71-3, part n 341134) has been activated through hydrogenation of the coordinated diene. After that the catalyst has been completely dissolved in the organic solvent (methanol-d<sub>4</sub>), the substrate has been added, the NMR tube has been pressurized with 8 bar of hydrogen enriched in the para isomer (92% enrichment) and the reaction has been started by shaking the NMR tube for 5s at 350K.

In the phase extraction experiments, the Rh(I) catalyst has been activated in 30ul of ethanol, then, after that the complex has been dissolved, a chloroform solution of the substrate (240mM) has been added and the hydrogenation with parahydrogen has been carried out as reported above. More experimental details are described in the S.I.

Magnetic field cycle has been applied using a mu-metal chamber (triple shield) that allows to shield the earth's magnetic field and to obtain 25nT in the most shielded part of the chamber. A coil fed with current (Agilent arbitrary waveform generator 33220A) electronically controlled by a custom written function has been placed into the shield. The magnetic field cycle device has been provided by Aspect Imaging (IL).

For enzymatic reaction experiment, an aqueous solution (1ml) of 1mg of LDH, (about 600U) (Sigma-Aldrich L-Lactic Dehydrogenase from rabbit muscle part n.1254-25KU) NAD<sup>+</sup> (1mM, Sigma-Aldrich B-nicotinamide Adenine dinucleotide N7004) and NADH (0.5mM Sigma-Aldrich B-NAD reduced form disodium N6660-15VL) has been prepared in HEPES

buffer. The and sodium pyruvate (1mM) in a buffer medium (HEPES 40mM) have been used. More details about the experiments with cells lysate in the S.I. The concentration of the hyperpolarized [ $^{13}\text{C}$ ]lactate in the aqueous mixture, after dilution, was  $4\pm 1$  mM, the total volume was 1.5ml.

All the NMR spectra have been acquired using a 14T Bruker Avance NMR spectrometer.

Parahydrogen has been obtained using the Bruker ParaHydrogen Generator (BPHG).

## Acknowledgements

The Italian Association for Cancer Research (AIRC, 2015 TRIDEO call), Regione Piemonte (PAR FSC 2007/13) and Bracco Imaging (through a contract with IBB-CNR) are gratefully acknowledged for financial support.

We acknowledge the support and advices from Aspect Imaging scientists (Yoram Cohen and Peter Bendel).

Prof. Mario Chiesa and Dr. Elena Morra (University of Torino, Dept. of Chemistry) are gratefully acknowledged for the acquisition and discussion of the EPR spectra.

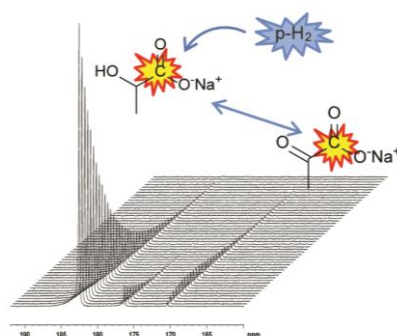
**Keywords:** hyperpolarization, Lactate dehydrogenase, metabolism, NMR spectroscopy, pyruvate.

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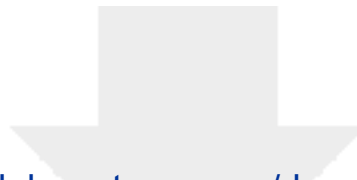
The hyperpolarization method ParaHydrogen Induced Polarization by means of Side Arm Hydrogenation has allowed to obtain a bio-compatible aqueous solution of  $^{13}\text{C}$ -MR hyperpolarized L-[1- $^{13}\text{C}$ ]lactate. The metabolic conversion of the hyperpolarized product into [1- $^{13}\text{C}$ ]pyruvate, catalyzed by the enzyme lactate dehydrogenase, has been observed by acquiring a series of  $^{13}\text{C}$ -NMR spectra (see figure).



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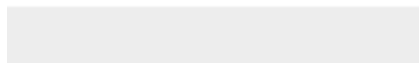
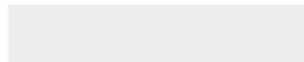
$^{13}\text{C}$ -MR Hyperpolarization of Lactate using ParaHydrogen and metabolic transformation in vitro.



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